Plant Host Effect on the Development of *Heliothis virescens* F. (Lepidoptera: Noctuidae)

CARLOS A. BLANCO,[1,2] ANTONIO P. TERÁN-VARGAS,[3] CRAIG A. ABEL,[1] MARIBEL PORTILLA,[4] M. GUADALUPE ROJAS,[4] JUAN A. MORALES-RAMOS,[4] AND GORDON L. SNODGRASS[1]

Environ. Entomol. 37(6): 1538–1547 (2008)

ABSTRACT *Heliothis virescens* F. is an important polyphagous pest that can develop on >100 plant species, including 20 economic crops. Populations of this insect are believed to be locally maintained on a few crops and weed hosts in Washington County, MS. To find the intrinsic value of these plants for the development of *H. virescens* populations, we fed different laboratory and wild colonies with fresh and lyophilized plant tissue under a constant temperature. Development time of this insect under laboratory conditions varied up to 10 d between plant hosts and was dependent on the type of plant tissue provided: fresh or lyophilized. Life table parameters such as net reproductive rate, finite rate of increase, and generation time indicated that *Trifolium repens*, a wild host growing around agricultural fields year round, could be one of the most suitable local plant hosts for the development of *H. virescens.* Two species of *Geranium*, previously reported as the source of the first *H. virescens* generation in the region, had lower intrinsic value as a food source than did *T. repens. Gossyipium hirsutum*, perhaps the most important crop source of *H. virescens* in the region, produced low net reproductive rate and finite rate of increase parameters. Sampling conducted in agricultural fields during 2006 and 2007 found no larvae on the above mentioned wild hosts as it was previously reported. Results indicated that *H. virescens* populations in this region were not supported by the wild plant species growing around agricultural fields during the time when the survey took place.

KEY WORDS tobacco budworm, generation time, net reproductive rate, finite rate of increase, plant host abundance

Heliothis virescens F. (Lepidoptera: Noctuidae) (tobacco budworm) is an important pest of >19 crops and has been reported to feed on at least 80 wild plants (Blanco et al. 2007). This insect was considered one of the most important cotton (*Gossypium hirsutum* L.) pests in the delta region of Mississippi (Hardee et al. 2001). Recently the effective control of *H. virescens* with *Bacillus thuringiensis* (Berliner)–expressing cotton (transgenic *Bt* cotton) has greatly reduced its importance in fields where this agricultural technology has been adopted.

The constant exposure of the tobacco budworm to *Bt* cotton may induce its resistance to *B. thuringiensis.* This insect has already shown its ability to become resistant to a wide range of synthetic insecticides (Sparks 1981, Luttrell et al. 1987, Hardee et al. 2001, Terán Vargas et al. 2005). To prevent the development of resistance to *Bt* cotton, the U.S. Environmental Protection Agency has required growers to set aside refuges of non-*Bt* cotton that can serve as tobacco budworm-producing areas where *B. thuringiensis–susceptible moths can develop and mate with B. thuringiensis*–resistant moths that might develop in *Bt* cotton (Matten and Reynolds 2003). This successful strategy takes into consideration the possibility that other crops and weeds might provide sources of *B. thuringiensis–susceptible moths, in addition to those produced in refuges.*

In Washington County, MS, the agricultural landscape once dominated by cotton is now a more diverse agroecosystem composed primarily of corn (*Zea mays* L.), rice (*Oryza sativa* L.), soybeans (*Glycine max* L.), and cotton, of which only the latter has the potential of hosting moderate densities of *H. virescens* in recent years (Blanco et al. 2007). The adoption of *Bt* cotton is common in this area, potentially reducing the capacity for producing large numbers of tobacco budworm moths. Evidence for the local decline of *H. virescens* populations includes (1) the constantly decreasing number of males captured in pheromone traps in the area since the introduction of *Bt* cotton (Blanco et al. 2005, Adamczyk and Hubbard 2006), (2)

Mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture or The Instituto Nacional de Investigaciones Agrícolas y Forestales (INIFAP).

[1] USDA–ARS, Southern Insect Management Research Unit, Stoneville, MS 38776.

[2] Corresponding author, e-mail: carlos.blanco@ars.usda.gov.

[3] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. Campo Experimental Sur de Tamaulipas, Cuauhtémoc, TAM 89610, Mexico.

[4] USDA–ARS, Biological Control of Pests Research Unit, Stoneville, MS 38776.

Table 1. Presence of *H. virescens* generations and phenology of its host plants in Washington County, MI, in 2006–2007

Plant host	Collection time	Plant phenology											
		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Abutilon theophrasti	April[b]				Fo	Fl	Se	Se					
Cicer arietinuma[a]	June[b,c]			De	De	De	De	De	De	De	De	De	
Geranium carolinianum	April[b,c]		Fo	Fo	Fl	Se	Se						
Geranium dissectum	April[b,c]	Fo	Fo	Fl	Se	Se	Se						Fo
Gossypium hirsutum	June[b,c]					Fo	Fl	Fl	Fl	Fl	Se		
Ipomoea sp.	June[b]				Fo	Fo	Fo	Fl	Fl	Se	Se		
Medicago arabica	April[b,c]	Fo	Fo	Fl	Fl	Fl	Fo				Fo	Fo	Fo
Paulownia tomentosa	June[c]		Fl	Fo	Fo	Fo	Fo	Fo	Fo	Se	Se		
Trifolium repens	April[b,c]	Fo	Fo	Fl	Fl	Fl	Fo	Fo	Fo	Fo	Fo	Fo	Fo
Presence of *Heliothis virescens* generations					First		Second	Third			4rd		

[a] *C. arietinum* can be planted at different times and its phenology will depend on planting date.
[b] Fresh tissue experiment.
[c] Lyophilized tissue experiment.
De, depending on planting date; Fo, foliage development; Fl, flowering; Se, senescence.

the reduction of *H. virescens* larvae found on wild hosts around agricultural field margins (Stadelbacher et al. 1986 [98,000–403,000 larvae/ha in 1981], Snodgrass et al. 1991 [62 larvae/ha in 1986], C.A.B., unpublished data [0 larvae/ha in 2004–2007]), and (3) the sporadic and low moth emergence on cotton and *Abutilon theophrasti* (Medikus) (Blanco et al. 2007).

The goals of this study were (1) to document the intrinsic capacity of *H. virescens* to develop on suitable plant hosts of Washington County, MS, and (2) to re-evaluate the current use of wild hosts by tobacco budworms near agricultural areas in Washington County, MS.

Materials and Methods

***Heliothis virescens* Plant Hosts, Their Phenology, and Density Around Agricultural Fields.** Early-season plant hosts (Carolina geranium [*Geranium carolinianum* L., Geraniaceae], cutleaf geranium [*G. dissectum* L.], white clover [*Trifolium repens* L., Fabaceae], and spotted medicago [*Medicago arabica* L. Huds., Fabaceae]) and mid-season plant hosts (velvetleaf [*Abutilon theophrasti* Medik, Malvaceae] and common morning glory [*Ipomoea* sp., Convolvulaceae]) were obtained from agricultural field margins in Washington County, MS, during April to August 2005 and 2006. Cotton (*Gossypium hirsutum* L., Malvaceae, DPL491 variety) and garbanzo (*Cicer arietinum* L., Fabaceae, Sierra variety) were planted the second week of May in experimental plots in 2005 and 2006. Foliage of the princess tree [*Paulownia tomentosa* (Thund.) Steud., Paulowniaceae], a season-long host plant, was obtained from trees planted in a private residence in 2002. Plant hosts were collected after the first local capture of tobacco budworm males in pheromone traps (early-season hosts, beginning in the second week of April) and after the subsequent pheromone capture peak activity (mid-season hosts, beginning in the second week of June; Table 1).

Twenty-five line intersect transects (Southwood 1978) of 25 m each along the margins of agricultural fields were performed to estimate percentage weed coverage of the plant species described in Table 1. These monthly surveys were conducted between March 2006 and November 2007 in 25 randomly selected places in Washington County, MS, and represented ≤8% of the length of an agricultural field (Table 2). During peak tobacco budworm moth activity (as indicated by the local pheromone trap captures), nearly solid stands of wild hosts of at least 3 m in any direction were sampled with a 28-cm sweep net (2 sweeps/m) on a weekly basis. Foliage harvested for the experiments described below was thoroughly inspected for larvae. Cotton and garbanzo used for the experiments described below were evaluated by shaking plants into a 1-m drop cloth. Larvae were placed on artificial diet (Blanco et al. 2008) and reared to adults for species identification (Brazzel et al. 1953).

Tobacco Budworm Colonies. Three colonies were used for this study: (1) a colony established in 1971 from larvae collected from wild hosts in Washington County, MS (referred to as ARS), maintained in the USDA–ARS facility in Stoneville, MS, (2) a colony established in 2006 from 250 single pair matings between ARS moths and moths collected from various plant hosts in Mississippi, North Carolina, Louisiana, and Texas (referred to as field), aiming to represent ample feral genetic diversity while conserving its adaptability to laboratory conditions, and (3) a colony of unknown origin maintained at the Monsanto Company facility in Union City, TN (referred to as Monsanto). These three colonies were fed the same artificial diet (Blanco et al. 2008) for at least a generation before the initiation of the study.

Life Table Studies Using Plant Tissue–Incorporated Diet. Plant tissue (Table 1) was harvested in 2006 every 1–3 d to represent different phenological stages, from early foliage development to seed maturation. Plant tissue was placed in freezer bags (gallon size; Ziploc, Crawfordville, IN), frozen at −80°C, and lyophilized (Freezemobile 35XL; The VirTis Company, Gardiner, NY). Tissue drying time varied among plant species. The dried tissue was milled (Laboratory Mill 3600; Perten Instruments, Huddinge, Sweden) to a fine powder. Lyophilized tissues were mixed into a

Table 2. Percent weed coverage and maintenance (herbicide-treated and/or mowed) of field margins around agricultural fields in Washington County, MI, 2006–2007

Date	*Geranium carolinianum*	*Geranium dissectum*	*Ipomoea* sp.	*Medicago arabica*	*Trifolium repens*	Percent of recently treated[a] or mowed fields
16 Mar. 2006	0.05	2.1	0	0.22	3.1	22
7 Apr. 2006	0.34	2.9	0	0.04	2.78	30
5 May 2006	0.06	0.21	0	0.04	1.04	8
8 June 2006	0	0.06	0	0	4.44	28
18 July 2006	0	0	0.53	0	2.58	40
10 Aug. 2006	0	0	0.12	0	0.78	0
18 Sept. 2006	0	0	0.03	0	1.22	0
3 Oct. 2006	0	0	0	0.16	1.65	0
6 Nov. 2006	0	0	0	0.82	3.08	0
14 Dec. 2006	0	6.64	0	0.99	5.17	8
6 Jan. 2007	0	1.2	0	2.22	5.66	0
11 Feb. 2007	0	0.22	0	0.85	4.93	0
13 Mar. 2007	0.24	3.26	0	2.97	7.28	8
8 Apr. 2007	0.26	3.3	0	0.03	1.8	8
11 May 2007	0.53	3.2	0.08	0	0.88	10
5 June 2007	0.37	0.11	0.048	0	3.12	24
6 July 2007	0	0	0.06	0	10.2	4
3 Aug. 2007	0	0	0.37	0	2.66	2
7 Sept. 2007	0	0	0.01	0	0.003	8
4 Oct. 2007	0	0	1.11	0	0.08	2
16 Nov. 2007	0	0.08	0	0	0.49	48

[a] Herbicides were used to control vegetation on fields.

common batch and were stored frozen (−80°C) until incorporation into diets. Diets consisted of 168 g of lyophilized plant tissue incorporated in a solution of 2,200 ml of distilled water containing 35 g of dissolved agar (AEP Colloids, Saratoga Springs, NY), 2.8 g of chlortetracycline (No. 6611B; Fort Dodge Animal Health, Ft. Dodge, IA), 2.8 g of methyl-p-hydroxybenzoate (No. 102341; MP Biomedicals, Solon, OH), 2.8 g of sorbic acid (No. 102937; MP Biomedicals), and 15 g of vitamin mix (DSM Nutritional Products, Alberta, Canada). Sets of two control diets, prepared simultaneously with the lyophilized plant tissue, followed the same process: (1) incorporating wheat germ instead of plant tissue, a diet that produces suboptimal *H. virescens* growth rate, and (2) insect artificial diet that provides optimal *H. virescens* growth rate (Blanco et al. 2008). Diets were poured (≈15 ml) into 37-ml plastic cups (No. T-125; Solo, Urbana, IL) and closed with a cardboard lid. Diets for the F_1 generation were prepared on different days because of the asynchronous emergence of F_1 neonates from different treatments. One F_0 neonate (≤16 h old) per colony infested each of the 150 cups per treatment for each generation tested. Larvae in cups were held as previously described, and developmental parameters were checked daily until the last insect died. Moths emerging from each treatment/insect colony were paired (≤18 pairs, depending on moth availability) in 500-ml containers (No. 42505L1; Consolidated Plastic, Twinsburg, OH) with free access to 10% sucrose solution in a 37-ml cup with a paper tissue (Kleenex, Roswell, GA) stuffed on it. Containers were capped with cloth (Batist; Zweigart, Piscataway, NJ) as an oviposition medium and maintained in incubators at 27 ± 0.4°C, 75 ± 10% RH, and a photoperiod of 14:10 h (L:D). Cloths with eggs were removed daily, and the number of eggs was estimated. The eggs were placed in plastic bags (sandwich size; Ziploc) in the same incubators for larval hatching assessment. Moths were maintained in containers until death. A random sample of the neonates (150 F_1 generation) produced by pairs of each treatment were used to repeat the process. Diet for F_1 neonates per treatment was inoculated on different days.

Life Table Studies Using Fresh Plant Tissue. Host plant tissues (Table 1) were collected every 1–3 d in 2005. Fresh plant tissue was rinsed, disinfected with a 1% sodium hypochlorite (regular bleach; Clorox, Oakland, CA.) solution for 10 min, triple rinsed with distilled water, and centrifuged in a salad spinner (Zyliss, Zurich, Switzerland) to remove excess water. Plant tissue per treatment was infested with 300 (≤16 h old) neonates from ARS and Monsanto colonies, maintained in 37-ml plastic cups closed with plastic lids (No. PL1; Solo). Fresh plant tissue was added or changed as needed. A set of 300 neonates per colony was also placed on insect artificial diet. Larval developmental parameters were checked daily until the last insect died. This process was repeated at least two times to obtain moths from each treatment.

Calculation of Reproductive Parameters and Statistical Analysis. The net reproductive rate (R_o), development time, net fertility, intrinsic capacity for increase (r_m), finite rate of increase (λ), doubling time, female longevity, and eggs produced were constructed using Lotka's equation (Carey 1993, Krebs 2001) and arithmetical calculations from a cohort of ≤18 pairs (1 ♀:1 ♂) per treatment per colony per generation. A data set consisting of 20 observations was generated for each treatment and generation of all the colonies by using a modification of the Tukey's Jackknife technique (Efron 1982). The jackknife

method consisted of sequentially deleting a single observation from the life table data, calculating the life table parameters, restoring the original data, deleting the next observation, and repeating this procedure until all data units were deleted and restored (Efron 1982, Meyer et al. 1986, Potvin and Roff 1993). Instead of deleting a single observation from the life table data, a set of five observations (none repeated) were randomly selected, deleted, and restored to generate the life table parameters. This procedure was repeated 20 times to generate the 20 observations for each life table. Demographic parameters (means ± SE) were analyzed using a one-way analysis of variance (ANOVA; ANOVA for uneven replications) by the general lineal model (GLM) procedure of SAS (9.1). Differences between least square means for all variables for each treatment and for each generation (calculated by the GLM procedure) were evaluated by the Tukey-Kramer's honestly significant difference (HSD) test.

Chemical Analysis of Fresh and Lyophilized Plant Tissue. Protein content of fresh and lyophilized cotton, princess tree, and garbanzo plant tissue was determined using the DC protein assay (No. 500-0116; Bio-Rad, Hercules, CA). Fresh collected leaves, stems, and young cotton squares were equally divided in two subsamples. One of the subsamples was lyophilized as described above, and the other subsample was stored at 5°C under dark conditions for 24 h to wait for the lyophilized subsample to be ready. Weights, before and after lyophilization, were taken to determine water content. Five hundred milligrams of each of the fresh samples was prepared by cutting (with fine scissors) pieces of leaves, stems, and/or squares. Equivalence based on dry weight was done to determine the sample size of 35 mg of the lyophilized material. Each sample was individually weighed (PB303-S Mettler Toledo; Fisher Science, Suwanee, GA) into 20-ml scintillation vials (No. 3002–1; Fisher Science), diluted to a 1:10 concentration with an aqueous 0.9% sodium chloride (No. S-5886; Sigma-Aldrich, St. Louis, MO) solution, and mixed using a Tissue-Trearor (model 985–370; BioSpec Products, Daigger, Vernon Hills, IL) for 5 min each. The samples were allowed to sit at room temperature for 5 min. Fifty microliters of each sample was individually transferred to a 15.0 by 1.0-cm glass vial, and 250 μl of reagent A (Bio-Rad kit) was added to each tube before it was vortexed for 1 min using a Thermolyne, Maxi Mix II (model M37615; Barnstead/ThermoLyne, Duburque, IA). Then, 250 μl of reagent B (Bio-Rad kit) was added to each tube and vortexed. Tubes with samples were allowed to react for 15 min. After reaction time elapsed, samples were transferred to 2.5-ml square disposable optical cells (P. No. FX805A; Daigger, Vernon Hills, IL), and their absorbance at 750 nm was read against a reference sample, using a PC Scanning Spectrometer, UV-VIS AUTO (model 2600; LaboMed, Culver City, CA). Percent protein was calculated based on the response of standard proteins to the DC protein test. Reference samples were prepared using 50 μl of 0.9% sodium chloride and reacted as plant tissue samples. Three separate analyses per 500 mg of fresh tissue and 35 mg of lyophilized plant tissue and their corresponding references were used.

Terpene contents of fresh and lyophilized plant material were determined following the method reported by Morales-Ramos and Rojas (2003). As above, the amounts of samples to be analyzed were determined on a dry weight basis. Fresh samples (500 mg per plant tissue) and lyophilized plant material (124 mg cotton, 110 mg princess tree, and 124 mg garbanzo) were weighed into 20-ml scintillation vials, and 2 ml of anhydrous ether (P. No. 9244–01; JT Baker, VWR, West Chester, PA) were added to each vial. The vials were tightly closed and held under a flow hood for 1 h. After this period of time, the solvent of each sample was transferred to a clean vial. Each sample was washed twice with 5 ml of ether, and both washes were combined with the ether from the original sample. Vials containing the washes were concentrated to dryness under a stream of ultra pure nitrogen. Each sample was mixed with 500 μl of hexane (No. 9262–02; JT Baker, VWR):ethyl acetate (No. 100–1; B&J Brand, VWR), 9:1 solution and then transferred to a Miniuniprep, 0.45-μm pore size nylon filter (No. UN203NPUNYL; Whatman, VWR). Once the samples were filtered, the vials were directly analyzed by GC-MS with a Hewlett-Packard, 6890N GC, equipped with a 5975 inert XL mass selective detector (MSD), a 7683 series autosampler, and an Agilent 19091S-433, HP-5MS, 5% phenyl methyl siloxane capillary column with a 250-μm diameter and 0.25-μm film thickness (Agilent Technologies, Palo Alto, CA). A 1-μl sample was injected at 250°C with a split ratio of 50:1 with a total flow of 53.6 ml. Oven temperature was programmed to ramp from 60 to 170°C at 15°C ml/min and then to 250°C at 5°C/min, with a final temperature hold of 5 min. Data were set to be acquired at 280°C with a 3-min solvent delay and a resulting electron multiplier (EM) voltage of 1,035.5 V. Scan parameters were as follows: low mass = 50.0 m/z; high mass = 550.0 m/z; threshold = 150 m/z (m/z = mass to charge ratio); sample no., = 2; A/D samples = 4; plot 2. Mass spectral (MS) zones settings were MS Quad 150°C and MS source = 230°C. Chromatogram peaks were identified by comparing retention times and mass spectra to those reported in the Wiley's NIST 2005 mass spectral library (Wiley Registry 2005) (Agilent Technologies). Two extractions per sample tissue were done. Data were analyzed by *t*-test (SAS Institute 2001).

Results and Discussion

Results indicated differences in the abundance of plant hosts, their influence on tobacco budworm development, and intrinsic differences between *H. virescens* colonies.

Plant Hosts. The most important plant species in terms of presence, abundance, and appropriate phenological stage for the development of feral *H. virescens* was white clover (*T. repens;* Tables 1 and 2), a reported plant host (Brazzel et al. 1953). This plant was actively growing during all the pheromone trap

capture–indicated peaks of this insect pest. However, no tobacco budworm larvae were found on this plant host during the 2-yr survey. Its potential value as a *H. virescens* host was indicated by the consistently high parameters such as one of the shortest time between generations, highest net reproductive rate (R_o), which is the mean number of female offspring produced by each female during its entire lifetime, the finite rate of increase, and shortest generation doubling time. These values were obtained from two subsequent generations under laboratory conditions (Table 3; Fig. 1). The three tobacco budworm colonies developed ≈8 d faster on lyophilized *T. repens* diet (and *M. arabica*) than on the other early-season hosts (e.g., *Geranium* spp.), but this trend was not consistent when two of these colonies were fed fresh plant tissue. Tobacco budworms developed ≈3 d faster on fresh *G. dissectum* than in the rest of the plant hosts (Table 4).

Geranium spp. have been reported to be important hosts of tobacco budworms in Washington County, MS (Stadelbacher et al. 1986, Snodgrass et al. 1991). However, we found no *H. virescens* larvae on either species over the 2-yr survey. *Geranium carolinianum* (data not shown), the least abundant of the two species (Table 2), was the only host on which two sequential generations of tobacco budworm developed with two of the insect colonies. The Monsanto colony was capable of developing only one generation on this plant species (Table 3), because the moths did not produce viable F_1 offspring after being fed *G. carolinianum* as larvae. Females fed these plants began producing females later (≈13 d) than the females fed the other early-season host (*T. repens*) in the F_0 generation (Fig. 1). The life table parameters obtained when feeding tobacco budworms fresh *Geranium* tissue were not consistent among two insect colonies. Slightly better parameters were obtained with the ARS colony–fed fresh *G. carolinianum*, whereas *G. dissectum* produced better values with the Monsanto colony (Table 4).

The other naturally occurring plant hosts were not good or consistent plant hosts. *Abutilon theophrasti*, despite being considered a serious weed in the region and a good host for tobacco budworm (Stadelbacher 1981, Stadelbacher et al. 1986, Blanco et al. 2007), was not present around agricultural fields. Two different insect colonies, fed fresh foliage of this plant in three different attempts per colony, were not able to complete development under laboratory conditions (life table parameters, as calculated with the other plant hosts, were not produced with *A. theophrasti;* therefore, information is not presented). Because this plant growing under natural conditions has very high densities of whiteflies and heteropterans (*Niesthrea* sp.), it was difficult to produce lyophilized tissue or feed larvae with fresh tissue free of insects. A feral tobacco budworm generation can develop on this plant in the field, but this insect does not feed on or develop consistently on this plant year after year (Blanco et al. 2007); *A. theophrasti* is only suitable for one generation and then senesces. Similarly, lyophilized *M. arabica* was not a good host for two of the three insect colonies (Table 3), but it was for the Monsanto colony when it was used as fresh tissue (Table 4). No *H. virescens* larvae were found on these plants during the 2-yr survey. *Paulownia tomentosa*, an introduced and nonabundant species in the region, belonging to the same plant family as cotton (Malvaceae), produced intermediate-low values in two subsequent generations with two insect colonies (Table 3). Survey of princess trees in Washington County, MS, during the last 6 yr detected an infestation of tobacco budworm on this plant in only 1 yr (last generation, 2004). We tried three times to grow ARS and Monsanto *H. virescens* colonies on *Ipomoea* sp., without success. Therefore, results from this plant host are not presented.

Cicer arietinum is not a wild host of tobacco budworm, but it has shown its capability for having high densities of this insect under field conditions generation after generation and year after year (Blanco et al. 2007). This plant produced also some of the best life table parameters (short generation time, intermediate net reproductive rate, and finite rate of increase values and doubling time values) with two subsequent generations of two insect colonies. Females production began a few days earlier (Fig. 1) on this plant, and this production was one of the highest of all treatments (Table 3). However, again, great differences were found between insect colonies and especially with the type of tissue fed to larvae. Lyophilized tissue produced good insect development while fresh tissue produced no development after three attempts per colony at different plant phenological stages (Table 4). Presence of *H. virescens* on garbanzo plots was consistent for each local feral generation. Larval densities ranged from 500 (first generation in 2006) to 95,000 (third generation in 2005) larvae/ha, indicating that tobacco budworm moths were present in the environment when this study was conducted.

Gossypium hirsutum produced mixed results when larvae from the three insect colonies were reared on it. High parameter values (short generation time, highest net reproductive rate, finite rate of increase, and shortest doubling time; Table 3; Fig. 1) were produced with the first generation of the ARS colony. Development only occurred in one generation of the field colony, and no development occurred with the Monsanto colony when fed lyophilized tissue. The lowest life table parameters were obtained when the ARS colony was fed fresh cotton tissue, and intermediate values were obtained with the Monsanto colony–fed fresh cotton (Table 4). There was a difference of ≈11 d in the development of a generation between late season hosts such as cotton and white clover (Tables 3 and 4).

Overall, plant hosts belonging to the Fabaceae family (*C. arietinum*, *T. repens*, and *M. arabica*) were better tobacco budworm plant hosts that those species belonging to the Malvaceae (*G. hirsutum* and *A. theophrasti*) or Geraniaceae (geraniums) families. Significant ($P < 0.0001$) differences were found in time between generations ($F = 285$, df = 6,273 for F_0, $F = 97$, df = 5,194 for F_1), net reproductive rate ($F = 70$, df = 6,273 for F_0, $F = 14$, df = 5,194 for F_1), finite rate

Table 3. Effect of diets made with different lyophilized plant host tissue on the development of two sequential generations of three different *H. virescens* laboratory colonies

Host	Generation time (G)	Net reproductive rate (R_o)	Finite rate of increase (λ)	Doubling time (DT)	Generation time (G)	Net reproductive rate (R_o)	Finite rate of increase (λ)	Doubling time (DT)
	ARS colony initial (F_0) generation				ARS colony first (F_1) generation			
Geranium carolinianum	43.54 a (±0.02)	75.07 d (±1.55)	1.104 d (±0.0005)	6.99 a (±0.03)	41.62 a (±0.01)	84.28 d (±1.38)	1.112 e (±0.0004)	6.51 a (±0.02)
Gossypium hirsutum	37.17 c (±0.10)	146.92 a (±2.66)	1.143 a (±0.0006)	5.16 d (±0.02)	41.09 b (±0.02)	244.17 b (±5.34)	1.143 d (±0.0006)	5.18 b (±0.02)
Medicago arabica	33.61 e (±0.02)	57.95 e (±1.23)	1.128 c (±0.0008)	5.75 b (±0.03)	31.960 d (±0.02)	207.89 c (±4.71)	1.181 a (±0.0008)	4.15 e (±0.01)
Paulownia tomentosa	39.75 b (±0.01)	114.10 b (±1,27)	1.126 c (±0.0003)	5.81 b (±0.01)	38.82 c (±0.08)	231.09 b (±7.68)	1.150 c (±0.0009)	4.95 c (±0.02)
Trifolium repens	34.58 d (±0.01)	93.88 c (±1.96)	1.140 b (±0.0006)	5.28 c (±0.02)	38.76 c (±0.02)	294.88 a (±6.26)	1.157 b (±0.0006)	4.72 d (±0.01)
Artificial diet[a]	26.10 (±0.01)	292.77 (±7.02)	1.242 (±0.001)	3.18 (±0.01)	27.92 (±0.01)	304.55 (±4.18)	1.227 (±0.0004)	3.38 (±0.006)
F (and P) values	36270 (<0.0001)	362 (<0.0001)	624 (<0.0001)	697 (<0.0001)	7,948 (<0.0001)	203 (<0.0001)	1,190 (<0.0001)	1,464 (<0.0001)
	FIELD colony initial (F_0) generation				FIELD colony first (F_1) generation			
Cicer arietinum	26.14 e (±0.02)	171.99 a (±3.09)	1.217 a (±0.0008)	3.52 e (±0.01)	32.98 c (±0.03)	212.20 a (±5.88)	1.176 a (±0.0009)	4.27 c (±0.02)
Geranium carolinianum	44.23 b (±0.04)	119.24 b (±3.26)	1.113 d (±0.0007)	6.42 b (±0.04)	35.76 a (±0.03)	187.65 b (±5.04)	1.157 b (±0.0008)	4.74 b (±0.02)
Geranium dissectum	46.06 a (±0.06)	33.11 c (±1.42)	1.078 e (±0.001)	9.20 a (±0.12)	Data could not be generated with this host plant			
Gossypium hirsutum	41.39 c (±0.06)	131.48 b (±4.05)	1.124 c (±0.0009)	5.89 c (±0.04)	Data could not be generated with this host plant			
Trifolium repens	34.01 d (±0.01)	173.61 a (±3.36)	1.163 b (±0.0006)	4.57 d (±0.01)	35.29 b (±0.03)	102.26 c (±2.33)	1.139 c (±0.0007)	5.29 a (±0.02)
Artificial diet [a]	27.37 (±0.02)	205.38 (±3.75)	1.214 (±0.0007)	3.56 (±0.01)	26.24 (±0.02)	423.12 (±7.48)	1.259 (±0.0008)	3.00 (±0.009)
F (and P) values	32072 (<0.0001)	326 (<0.0001)	3,585 (<0.0001)	1,144 (<0.0001)	2,086 (<0.0001)	152 (<0.0001)	448 (<0.0001)	452 (<0.0001)
	Monsanto colony initial (F_0) generation				Monsanto colony first (F_1) generation			
Cicer arietinum	33.71 d (±0.06)	121.07 b (±4.38)	1.152 b (±0.001)	4.89 c (±0.03)	34.30 a (±0.04)	107.52 b (±2.50)	1.145 b (±0.0007)	5.09 b (±0.02)
Geranium carolinianum	45.20 b (±0.06)	13.97 d (±0.41)	1.059 d (±0.0007)	11.94 a (±0.13)	Data could not be generated with this host plant			
Paulownia tomentosa	47.39 a (±0.03)	51.06 c (±1.23)	1.086 c (±0.0006)	8.37 b (±0.05)	Data could not be generated with this host plant			
Trifolium repens	34.71 c (±0.02)	173.90 a (±3.30)	1.160 a (±0.0006)	4.66 c (±0.01)	34.31 a (±0.02)	198.57 a (±3.50)	1.166 a (±0.0006)	4.49 a (±0.01)
Wheat-germ[a]	33.84 (±0.15)	12.89 (±1.01)	1.075 (±0.004)	14.92 (±5.64)	31.84 (±0.02)	90.86 (±2.27)	1.151 (±0.0009)	4.90 (±0.02)
Artificial diet[a]	28.55 (±0.07)	151.76 (±2.68)	1.192 (±0.0007)	3.94 (±0.01)	25.94 (±0.02)	99.12 (±1.56)	1.193 (±0.0006)	3.91 (±0.01)
F (and P) values	20,342 (<0.0001)	640 (<0.0001)	3,326 (<0.0001)	1,909 (<0.0001)	0.13 (0.71)	446 (<0.0001)	438 (<0.0001)	397 (<0.0001)

Means (±SE of mean based on ANOVA) in columns by generation.

[a] Artificial diets not included in the ANOVA.

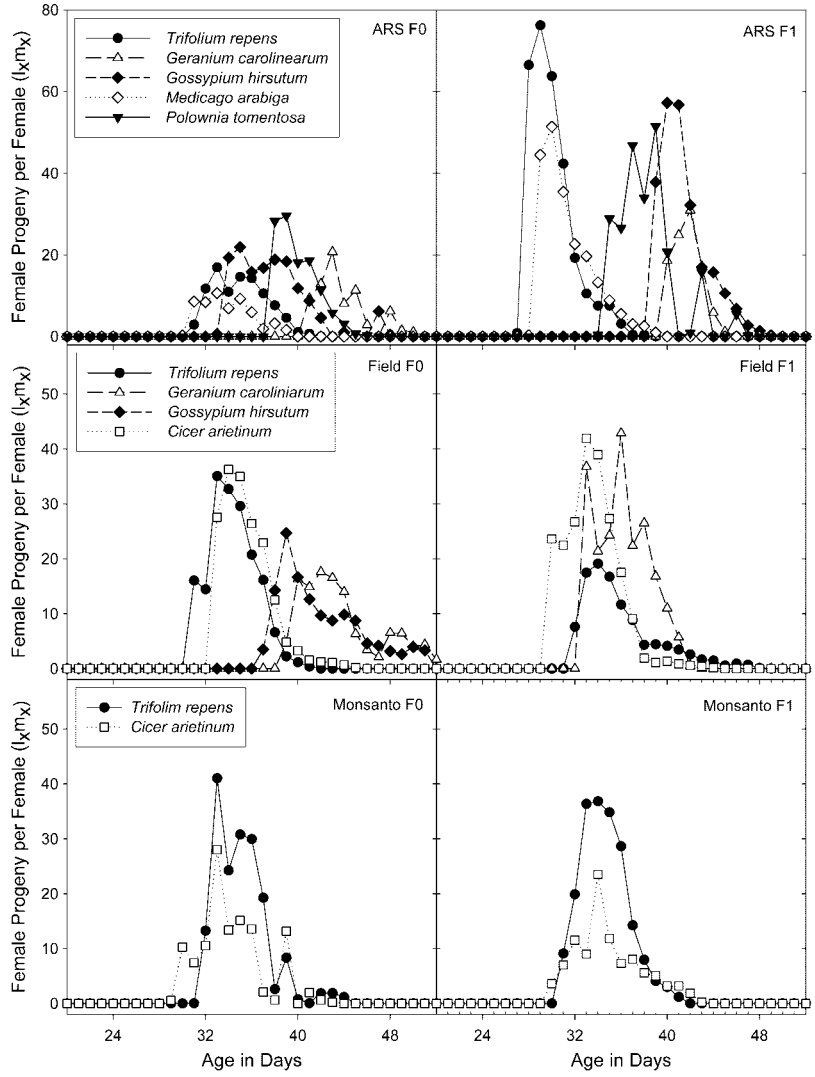

Fig. 1. Age-dependent net fecundity (lxmx) of three *Heliothis virescens* colonies developing in diets with lyophilized tissue of different host plants at start (F0) and after one generation (F1).

of increase ($F = 137$, df = 6,273 for F_0, $F = 34$, df = 5,194 for F_1), and doubling time ($F = 75$, df = 6,273 for F_0, $F = 29$, df = 5,194 for F_1) when the analyses were made comparing the lyophilized tissue data of the three colonies. Significant differences between colonies were also obtained on time between generations ($F = 26{,}273$, df = 3,76), net reproductive rate ($F = 1{,}072$, df = 3,76), finite rate of increase ($F = 978$, df = 3,76), and doubling time ($F = 244$, df = 3,76) when ARS and Monsanto colonies fed fresh tissue were compared. The differences obtained between lyophilized and fresh tissue could be explained in part by significant differences found in the plant tissue's chemical composition. Fresh cotton had 54% ($t = 10.1$, $P = 0.009$, df = 4), garbanzo had 57% ($t = 22.5$, $P = 0.002$, df = 4), and princess tree had 74% ($t = 55.3$, $P = 0.0003$, df = 4) more protein than lyophilized tissue. The amount of protein for optimal development of *H. virescens* is ≈3% (Guerra and Bhuiya 1977), which means that this insect is sensitive to high protein content in its diet. The terpenes content was variable between tissues of host plants. Lyophilized cotton had 17% ($t = 1.5$, $P = 0.36$, df = 2), lyophilized garbanzo had 43% ($t = 2.7$, $P = 0.22$, df = 2), and lyophilized princess tree had 57% ($t = 17.7$, $P = 0.03$, df = 2) more terpenes than fresh tissue. The changes in the protein concentration might have been the cause for the differences in development of the three insect colonies. The significant differences in the performance of tobacco budworm on the two types of diet indicates that the use of lyophilized tissue diet requires further work for a thorough understanding of its differences with fresh tissue and/or plants growing in the field and the development of tobacco budworm. However, its use could reduce variation in studies of larval development because large batches of lyophilized tissue rep-

Table 4. Effect of different fresh plant host tissues on the development of two colonies of *H. virescens*

Host	Generation time (G)	Net reproductive rate (R_o)	Finite rate of increase (λ)	Doubling time (DT)
		ARS colony initial (F_0) generation		
Geranium carolinianum	35.55 b (±0.03)	43.56 a (±0.63)	1.111 a (±0.0004)	6.53 c (±0.02)
Geranium dissectum	29.76 d (±0.02)	18.15 b (±0.34)	1.102 b (±0.0007)	7.13 bc (±0.04)
Gossypium hirsutum	43.37 a (±0.01)	9.28 c (±0.33)	1.052 d (±0.001)	13.78 a (±0.42)
Trifolium repens	32.85 c (±0.05)	19.01 b (±0.41)	1.093 c (±0.0008)	7.75 b (±0.06)
Artificial diet[a]	29.85 (±0.009)	247.35 (±2.81)	1.202 (±0.0004)	3.75 (±0.007)
F (and P) values	26273 (<0.0001)	1,072 (<0.0001)	978 (<0.0001)	244 (<0.0001)
		Monsanto colony initial (F_0) generation		
Geranium carolinianum	40.86 b (±1.77)	3.71 d (±0.40)	1.022 c (±0.007)	11.28 b (±3.08)
Geranium dissectum	31.30 d (±0.03)	23.20 c (±0.45)	1.105 a (±0.0007)	6.91 b (±0.04)
Gossypium hirsutum	47.71 a (±0.16)	33.89 a (±3.85)	1.074 b (±0.004)	10.02 b (±0.93)
Medicago arabica	36.23 c (±0.19)	1.643 d (±0.11)	1.011 c (±0.002)	32.12 a (±7.61)
Trifolium repens	35.91 c (±0.03)	27.89 b (±0.71)	1.096 a (±0.0008)	7.50 b (±0.06)
Artificial diet[a]	26.52 (±0.03)	138.31 (±2.77)	1.204 (±0.0009)	3.73 (±0.01)
F (and P) values	32 (<0.0001)	239 (<0.0001)	113 (<0.0001)	7 (<0.0001)

Means (±SE of mean based on ANOVA) in columns by generation by colony followed by the same letter are not significantly different.
[a] Artificial diet not included in the ANOVA.

resenting multiple plants could be harvested during the most suitable phenological stage and kept under appropriate conditions. Lyophilized tissue batches offer the opportunity for testing multiple sequential generations and providing the same nutritional value to each generation. It also reduces the intensive labor necessary for conducting studies with fresh tissue.

Insect Colonies. Two important observations were made with the performance of the insect colonies. First, during the initial generation (F_0), every colony was fed all the different plant hosts described in Table 1 and insect artificial diets. Two colonies performed well on *G. carolinianum* (ARS and field), an expected result because these two colonies share 50% of their genetic background. However, one of them (ARS) was not able to develop in *C. arietinum*, whereas the other "sibling" colony (field) was. Development on cotton occurred only in the first generation of the field colony, whereas the ARS colony completed both generations. This compared with the response of the third colony (Monsanto) that succumbed during the first generation exposed to lyophilized cotton tissue. The origin and genetic diversity of the tobacco budworm colonies used in this type of study is a factor to consider during the interpretation of results.

Second, there is a trend for obtaining greater similarity in parameters among treatments in the F_1 generation than in the initial F_0 generation (Table 3), showing the adaptation plasticity that this insect has when it encounters different hosts. This was particularly noticeable, for example, with the change that occurred between the F_0 and F_1 generations of the ARS colony fed *M. arabica.*

Heliothis virescens was very abundant in the Washington County, MS, area before 1981 (Stadelbacher 1981), but during 2001–2007, they were nearly absent in the wild hosts we studied and on cotton. Weed abundance has been greatly reduced by several agricultural changes that have occurred throughout the Mississippi River Delta since the mid-1990s. The extensive use of crops (cotton, corn, soybeans) that are resistant to the use of broad-spectrum herbicide(s) has greatly improved weed control in these crops. The widespread planting of corn and groups IV and V soybeans in March and April requires early-season weed destruction. Growers control winter and spring weeds by making mostly aerial applications of broad-spectrum herbicides in February and March. By the time cotton is planted (April–May), most of the weeds are restricted to marginal areas near roads, ditches, and fields. Even these marginal areas are frequently treated with the same broad-spectrum or selective herbicides aiming to reduce broadleaf weeds to diminish tarnished plant bug [*Lygus lineolaris* (Palisot de Beauvois)] populations (Snodgrass et al. 2006). Currently, tobacco budworm captures in pheromone traps also show decreasing numbers (Blanco et al. 2005, Adamczyk and Hubbard 2006), but in experimental *C. arietinum* plots, larval densities have reached up to 125,000/ha (Blanco et al. 2007) and, during this survey, 500–95,000 larvae/ha. Cotton and white clover seem to be the last plant hosts for the development of the last generation of tobacco budworm in ≈October and November. Cotton in this region is predominantly *Bacillus thuringiensis* expressing (*Bt* cotton) with a high efficacy against this pest. In those few non-*Bt* cotton areas, infestations of tobacco budworms are rarely seen (unpublished data), making this a host that produces very few insects, if any, for the build-up of the next generation. *Trifolium repens* representing ≤1% (Table 2) of the 3.5% of the agricultural landscape represented by marginal areas has a low potential for the build-up of the last *H. virescens* (overwintering) population during October and November that putatively gives rise to the next year's first tobacco budworm generation. Therefore, the possibility of a local plant host that we have not identified yet, and/or the recolonization of the Washington County, MS, area by migrating populations early in the year, might be the source of *H. virescens* captured in pheromone traps and /or early-season

garbanzo plots. Regardless of the source of local *H. virescens* colonization (immigration or local emergence) during the first generation of the year, let us assume that these moths simultaneously infest the early plant hosts (seven hosts with adequate plant phenology for the development of this insect; Table 1). The subsequent generation of moths emerging from these plants could have an asynchronous emergence pattern of up to 10 d between members of the Fabaceae family (*T. repens* and *M. arabica*) and *G. carolinianum*, which may indicate that moths emerging from plant species belonging to one plant family have a greater chance to mate among themselves (Fig. 1). A similar pattern of moth emergence and mating synchrony can be assumed for the second *H. virescens* generation. Four species of actively growing plant hosts (Table 1) coincide with the appropriate cotton phenology for tobacco budworm development (square formation). If first-generation moths simultaneously oviposit on these five hosts, the emergence of F_2 moths in this area could be asynchronous up to 10 d between cotton and white clover (Fig. 1).

This study presents unique information because we compared simultaneously the development of this important pest on different plant hosts under the same environmental conditions that otherwise do not occur in the field. It also produced complete life table parameters for the important plant hosts found in the Washington County, MS. As far as we could determine, these are the first life table studies done with the tobacco budworm. This information revisits the role of plant hosts in building local tobacco budworm populations.

Acknowledgments

We thank A. Petterson, A. Ellis, C. Roberts, G. Patterson, and A. Walters for help conducting this research. We thank N. Adams of Monsanto Company for the insect donations and L. Adams for providing the pheromone traps data. This manuscript was greatly improved by following the observations made by H. J. McAuslane and two anonymous reviewers to a prior draft.

References Cited

Adamczyk, J., Jr., and D. Hubbard. 2006. Changes in populations of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae) and *Helicoverpa zea* (Boddie) (Lepidoptera: Noctuidae) in the Mississippi Delta from 1986 to 2005 as indicated by adult male pheromone traps. J. Cotton Sci. 10: 155–160.

Blanco, C. A., M. Mullen, C. Abel, J. R. Bradley, P. Ellsworth, J. K. Greene, A. Herbert, R. Leonard, J. D. Lopez, Jr., R. Meagher, W. Moar, M. Parajulee, R. D. Parker, P. Roberts, J. Ruberson, R. Sprenkel, G. Studebaker, A. P. Terán, M. Williams, and J. Van Duyn. 2005. *Bacillus thuringiensis* Cry1Ac resistance monitoring program for the tobacco budworm and bollworm in 2004. Proceedings of the 2005 Beltwide Cotton Conference, 4–7 January 2005, New Orleans, LA.

Blanco, C. A., A. P. Terán-Vargas, J. D. López, Jr., J. V. Kauffman, and X. Wei. 2007. Densities of *Heliothis virescens* and *Helicoverpa zea* (Lepidoptera: Noctuidae) in three plant hosts. Fla. Entomol. 90: 742–750.

Blanco, C. A., M. Portilla, C. A. Abel, H. Winters, R. Ford, and D. Street. 2008. Soybean flour and wheat germ proportions in insect artificial diet and their effect on the growth rates of *Heliothis virescens* (F.) (Lepidoptera: Noctuidae). J. Ins. Sci. (in press).

Brazzel, J. R., C. Lincoln, L. D. Newson, F. J. Williams, J. S. Roussel, and G. Barnes. 1953. Bollworm and tobacco budworm as cotton pests in Louisiana and Arkansas. Louisiana Agric. Expt. Stn. Tech. Bull. 482.

Carey, F. G. 1993. Applied demography for biologists with special emphasis on insects. Oxford University Press. Oxford, United Kingdom.

Efron, B. 1982. The jackknife, the bootstrap, and other resampling plans. Soc. Ind. Appl. Math. Monograph Volume 38.

Guerra, A. A., and A. D. Bhuiya. 1977. Nutrition of the tobacco budworm: an economical larval diet for rearing. J. Econ. Entomol. 70: 568–570.

Hardee, D. D., L. C. Adams, and G. W. Elzen. 2001. Monitoring for changes in tolerance and resistance to insecticides in bollworm / tobacco budworm in Mississippi, 1996–1999. Southwest. Entomol. 26: 365–372.

Krebs, C. J. 2001. Ecology: the experimental analysis of distribution and abundance, 5th ed. Wesley Longman, San Francisco, CA.

Luttrell, R. G., R. T. Roush, A. Ali, J. S. Mink, M. R. Reid, and G. L. Snodgrass. 1987. Pyrethroid resistance in field populations of *H. virescens* (Lepidoptera: Noctuidae) in Mississippi in 1986. J. Econ. Entomol. 80: 985–989.

Matten, S. R., and A. Reynolds. 2003. Current resistance management requirements for Bt cotton in the United States. J. New Seeds. 5: 1317–178.

Meyer, J. S., C. G. Ingersoll, L. L. McDonald, and M. S. Boyce. 1986. Estimating uncertainty in population growth rates jackknife vs. bootstrap techniques. Ecology 67: 1156–1166.

Morales-Ramos, J. A., and M. G. Rojas. 2003. Formosan subterranean termite feeding preference as basis for bait matrix development (Isoptera: Rhinotermitidae). Sociobiology 41: 71–79.

Potvin, C., and D. A. Roff. 1993. Distribution-free and robust statistical methods: viable alternatives to parametric statistics? Ecology 74: 1617–1628.

SAS Institute. 2001. Version 9.1. SAS Institute, Cary, NC.

Snodgrass, G. L., E. A. Stadelbacher, and J. W. Smith. 1991. Distribution and abundance of early-season wild host plants and bollworm and tobacco budworm populations (Lepidoptera: Noctuidae) in an intensively cropped area of the Mid-Delta of Mississippi. J. Entomol. Sci. 26: 9–16.

Snodgrass, G. L., W. P. Scott, C. A. Abel, J. T. Rubbins, J. Gore, and D. D. Hardee. 2006. Suppression of tarnished plant bugs in cotton (Heteroptera: Miridae) in cotton by control of early season wild host plants with herbicides. Environ. Entomol. 35: 1417–1422.

Southwood, T.R.E. 1978. Ecological methods with particular reference to the study of insect populations, 2nd ed. Chapman & Hall, London, United Kingdom.

Sparks, T. C. 1981. Development of insecticide resistance in *Heliothis zea* and *Heliothis virescens* in North America. Bull. Entomol. Soc. Am. 27: 186–192.

Stadelbacher, E. A. 1981. Role of early-season wild and naturalized host plants in the buildup of the F_1 generation of *Heliothis zea* and *H. virescens* in the Delta of Mississippi. Environ. Entomol. 10: 766–770.

Stadelbacher, E. A., H. M. Graham, V. E. Harris, J. D. Lopez, J. R. Phillips, and S. H. Roach. 1986. *Heliothis* populations and wild host plants in the Southern U. S., pp. 54–74. *In* S. J. Jonson, E. G. King, and J. R. Bradley, Jr. (eds.). Theory and tactics of *Heliothis* population management.

Terán Vargas, A. P., J. C. Rodríguez, C. A. Blanco, J. L. Martínez Carrillo, J. Cibrian Tovar, H. Sánchez Arroyo, L. A. Rodríguez del Bosque, and D. Stanley. 2005. Bollgard cotton and resistance of the tobacco budworm (Lepidoptera: Noctuidae) to conventional insecticides in Southern Tamaulipas, Mexico. J. Econ. Entomol. 98: 2203–2209.

Received 25 January 2008; accepted 21 July 2008.